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Ultrasonic-associated extraction of water soluble polysaccharides from defatted Korean pine kernel

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Abstract: Experiments of ultrasonic-associated extraction and hot water extraction of water soluble polysaccharides from defatted Korean pine (*Pinus koraiensis*) were conducted in Northeast Forestry University, Harbin, China. The factors affecting extraction yield and content, such as extracting temperature, extracting time, the ratio of water to defatted kernel and concentration of ethanol were analyzed under specific condition. The optimal extracting parameters for ultrasound-associated extraction were determined as the ultrasonic temperature 70℃, the ratio of defatted kernel to water 1:20, the extracting time 40 min, and ethanol concentration 80%. Under such extraction conditions, the yield of water soluble polysaccharide was 3.65% and the average content of polysaccharide was 45.38% in the raw polysaccharides which gained in the experiment. Both extraction yield and content of polysaccharides extracted with ultrasound-associated extraction were higher than that with hot water extraction. The study demonstrates that ultrasound is a reliable and extremely effective tool for the fast extraction of water soluble polysaccharide of Korean pine kernel.

Key words: Ultrasonic; Defatted Korean pine kernel; Polysaccharides

Introduction

Ultrasonic extraction technology has been widely applied in extracting oil, polysaccharides, proteins, and flavonoids from plant, etc (Zhang *et al*. 2005; Li *et al*. 2006; Ma *et al*. 2006; Jiang *et al*. 2006). Research showed that this technology has great efficiency, uses less energy, and does not destroy the effective component of materials.

Polysaccharides have been proposed as the first biopolymers formed on Earth. They are classified on the basis of their main monosaccharide components and the sequences and linkages between them, as well as the anomeric configuration of linkages, the ring size (furanose or pyranose), the absolute configuration (D- or L-) and any other substituents present. Later work has shown that polysaccharides are involved in a number of important biochemical functions, such as cell-cell interaction and communication, attachment for infectious bacteria, viruses, toxins and hormones, to mention just a few (He *et al*. 2006). Up to now, however, the extraction of kernel polysaccharides of Korean pine (*Pinus koraiensis*) by ultrasound-assisted extraction technology has not been reported. The objective of the present study is to provide a simple method for extracting water soluble polysaccharides, and the influence of the different factors on extracting yield and content was also investigated.

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Materials and methods

Materials

Korean pine kernel was collected from Xiaoxing'an Mountains, Heilongjiang Province, China. All ultrasonic extractions were carried out by KQ Signature Ultrasonic Cleaner Model 250DB with digital timer, heat and power (Kunshan Ultrasonic Instruments Ltd, China). Electronic scale and electrothermal constant temperature blast drier were provided by Tianjin Test Instruments Ltd. High Speed Refrigerated Centrifuge (3K30) was provided by SIGMA Laborzetrifugen GmbH (Germany). Rotary evaporator (RE5203) was provided by Shanghai Yarong Biochemistry Instrument Factory. Ltd. and ethanol (AR) was provided by Tianjin Chemical Reagent Co., Inc.

Methods

Korean pine kernel was defatted with ethanol. Dried defatted kernel was suspended with distilled water, and then treated with ultrasonic wave as plan. The extractive was centrifuged (30 min, 5 000 rpm·min^{-1}), and the supernatant was concentrated by rotary evaporator. The residue was extracted with Sevag reagent (Normal butanol: Chloroform=1:4) three times to deproteinize. After removing the Sevag reagent, ethanol was added to the extract and kept at 4ºC overnight to precipitate polysaccharides. The precipitate was re-suspended and the procedure was repeated again. Finally, the precipitate was frozen at - 20 ℃ overnight and freeze-dried to obtain water soluble polysaccharides powder.

Total carbohydrate content was determined by the modified phenol-sulfuric acid method (Xu *et al*. 2005).

Results and analysis

Effect of extracting temperature on extraction yield

The effect of different ultrasonic temperature on extraction yield

was investigated at ultrasonic frequency of 40 kHz, ultrasonic time of 30 min, and the ratio of material to liquid of 1:10. The extraction yield and content of polysaccharides increased with the increase of temperature (Fig. 1). The yield of extraction and the content of polysaccharides at 80 ℃ increased by 1.64% and 2.60% compared with that at 60 ºC. The result showed that it was not necessary to keep on higher temperature during the extraction. The extraction temperature should be kept under 80 ºC

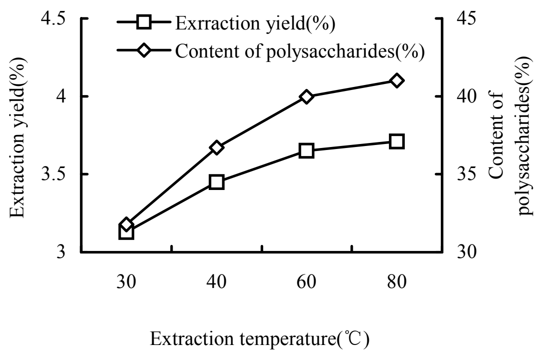

Fig. 1 Influence of temperature on extraction yield and content of polysaccharides

Effect of the ratio of water to defatted kernel on extraction yield

The effect of different ratio of water to defatted kernel on extraction yield was detected at the ultrasonic frequency of 40 kHz, ultrasonic time of 30 min, and the temperature of 40 ºC. Results showed that the total extraction yield of water soluble polysaccharides increased along with water increase. (Fig. 2) When the ratio of water to defatted kernel was used from 10:1 to 20:1, extraction yield reached up to the most value; when water was used from 20:1 to 40:1, the increasing scope of total polysaccharides extraction yield and content was smaller. Since a high ratio of water to kernel might increase the difficulty to the next extraction step, the ratio of kernel to water at 1:40 was enough, according to the experimental result.

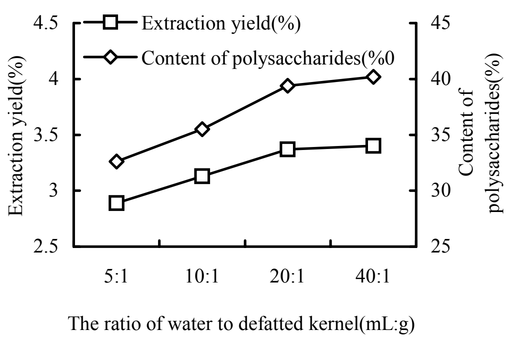

Fig. 2 Influence of the ratio of water to defatted kernel on extraction yield and content of polysaccharides

Effect of extracting time on extraction yield

The effect of different extracting time on extraction yield was detected at the ultrasonic frequency of 40 kHz, ratio of material to liquid of 1:10, and the temperature of 40 ºC. Results showed that, along with the increase in extraction time from 10 min to 80 min, the total polysaccharides content and extraction yield increased, but after the special time point of 40 min, the extraction yield no longer increased, and the content increase was very small (Fig. 3). Therefore, the experimental result demonstrates that water soluble polysaccharides can be extracted basically within 40 min under ultrasonic treatment.

Effect of ethanol concentration on extraction yield

The effect of different ethanol concentration on extraction yield and content of polysaccharides was detected at 40 kHz, 30 min ultrasonic time, the ratio of defatted kernel to water of 1:10, and temperature of 40 ºC. Results showed that the total polysaccharides extraction yield increased with the increase in ethanol concentration when ethanol concentration was lower than 80%. Extraction yield was highest at ethanol concentration of 80%, and then decreased appreciably with the increase in ethanol concentration (Fig. 4). When ethanol concentration was lower than 80 %, the increasing scope of polysaccharides content was bigger; when ethanol concentration was situated between 80 % and 90 %, the increasing scope of polysaccharides content was smaller.

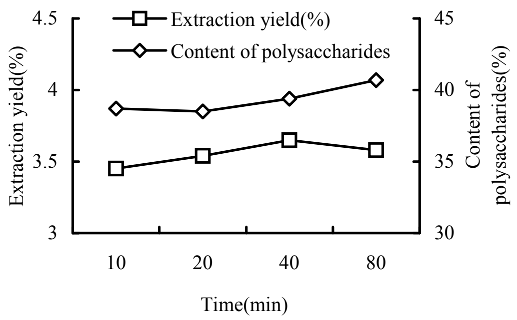

Fig. 3 Influence of extracting time on extraction yield and content of polysaccharides

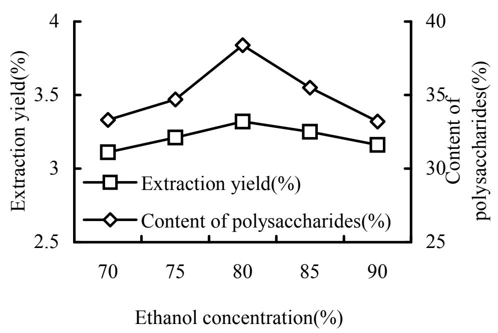

Fig. 4 Influence of ethanol concentration on extraction yield and content of polysaccharides

Determining the best condition of extraction

On the basis of experiment and analysis of single factor, the extracting temperature, extracting time, ratio of water to defatted kernel, and ethanol concentration were selected separately to set up three levels experiments (Table 1), and the influence of each factor on extraction yield and content of polysaccharides was observed. Design and results of ultrasonic extraction was shown in Table 1 and Table 2.

The optimum extracting conditions of polysaccharides were investigated by using orthogonal experimental design. It is oncluded from the result (Table 2) that the extraction yield was affected remarkably by parameters. The primary influencing factor, R value of extraction time was 0.390, followed by extraction temperature (0.280), the ratio of defatted pine kernel to water (0.264) and ethanol concentration (0.107) affected the yield relatively smaller in the four factors.

Table 1. Factors and levels of experiment

Levels	Factors			
	A Temperature (℃)	B Water：Defatted kernel（mL: g）	C Time (min)	D Ethanol concentration (%)
1	60	15：1	30	75
2	70	20：1	40	80
3	80	30：1	50	85

Table 2. Design and results of orthogonal experiment L_9 (3^4)

Test No.	A	B	C	D	Yield (%)	Content (%)
1	1	1	1	1	2.87	26.63
2	1	2	2	2	3.63	43.61
3	1	3	3	3	3.18	28.37
4	2	1	2	3	3.58	34.87
5	2	2	3	1	3.53	31.94
6	2	3	1	2	3.41	49.76
7	3	1	3	2	3.37	41.58
8	3	2	1	3	3.45	35.77
9	3	3	2	1	3.69	43.90
K1(Y)	3.227	3.273	3.243	3.363		
K2(Y)	3.507	3.537	3.633	3.470		
K3(Y)	3.503	3.427	3.360	3.403		
R(Y)	0.280	0.264	0.390	0.107		
K1(C)	32.87	34.36	37.387	34.157		
K2(C)	38.857	37.107	40.793	44.983		
K3(C)	40.417	40.677	33.963	33.003		
R(C)	7.547	6.317	6.83	11.98		

Table 3. Variance analysis (Yield)

Source of Variance	SS	F	F_α	Significance
A	0.155	2	9.118	*
B	0.105	2	6.176	
C	0.240	2	14.118	*
D	0.017	2	1.000	
Error	0.020	2		

Notes: A--Temperature; B--ultrasonic time; C--ratio of water to defatted kernel; D--ethanol concentration. All of freedom degree is 2, α=0.05. * represents significant difference.

Results also indicated that the optimum condition was $A_2B_2C_2D_2$, viz. extracting temperature was 70℃, the ratio of defatted pine kernel to water was 1:20, ultrasonic time 40 min, and ethanol concentration 80%. Under this optimal condition, the extraction rate of polysaccharides was 3.65%, and the content of polysaccharides was 45.38%. Variance analysis showed that Extracting temperature and time had remarkable influences on the extraction yield (Table 3).

Ultrasonic extraction compared with hot water extract method

As shown in Table 4, when defatted kernel was extracted for 30 min by ultrasonic, yield namely achieved 3.65 %, but the hot water extracted 30 min, 60 min, 120 min, yield in turn was 2.07%, 2.74%, 3.15%, and the content of polysaccharides also lower than that in ultrasonic extraction, and the obvious ultrasonic strengthening can achieve timesaving, high efficiency and energy saving.

Table 4. Ultrasound extraction compared with hot water extract method

Method	Time(min)		
	30	60	120
Ultrasonic extraction yield (%)	3.65	--	--
Ultrasonic extraction content (%)	45.38		
Hot water extraction yield (%)	2.07	2.74	3.15
Hot water extraction content (%)	30.19	32.64	35.58

Conclusion

Compared with hot water extraction, ultrasound extraction has many advantages, such as low temperature, solvent saving, time saving, high efficiency and high extraction yield. In this article the ultrasonic wave technology was used to extract the total water soluble polysaccharides in defatted Korean pine kernel. The optimal parameters were found to be as follows: extracting temperature of 70℃, the ratio of defatted pine kernel to water of 1:20, ultrasonic time 40 min, and ethanol concentration 80%. Under this optimal condition, the extraction rate of polysaccharides was 3.65%, and the content of polysaccharides was 45.38%. Both extraction yield and content of polysaccharides extracted with ultrasound-associated extraction were higher than that with hot water extraction. The results might provide a theoretic base for full utilization of water soluble polysaccharides in Korean pine kernel.

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